25. R. M. Perrin, K. Keegstra, N. V. Raikhel, data not shown.
26. Three motifs were found to be conserved among several α-1,2-fucosyltransferases, despite low overall homology. One ([IV]G[IV][HQ][VI]R..[DN]) has been described previously (*27*) (square brackets indicate that either of the indicated amino acids was found at the indicated position; dots indicate that three or more different amino acids were found at the indicated position). In addition, a second motif (D[EK][MQ][FI]F[CR][EQ].DQ) and a third region (G[LF]G[ND][RC][IL].[TS][LI]A[SA].[FW][LR][YF]A.[LQ]T[DG]R..[LA].[VI][DE]) were conserved (*29*).
27. C. Breton, R. Oriol, A. Inberty, *Glycobiology* **8**, 87 (1998).
28. J. E. Varner and L.-S. Lin, *Cell* **56**, 231 (1989).
29. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Dissociating Pain from Its Anticipation in the Human Brain

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The experience of pain is subjectively different from the fear and anxiety caused by threats of pain. Functional magnetic resonance imaging in healthy humans was applied to dissociate neural activation patterns associated with acute pain and its anticipation. Expectation of pain activated sites within the medial frontal lobe, insular cortex, and cerebellum distinct from, but close to, locations mediating pain experience itself. Anticipation of pain can in its own right cause mood changes and behavioral adaptations that exacerbate the suffering experienced by chronic pain patients. Selective manipulations of activity at these sites may offer therapeutic possibilities for treating chronic pain.

Intense, noxious stimulation leads to physiological, emotional, and behavioral changes of obvious adaptive significance (*1*). One is the experience of pain, which minimizes immediate harm by motivating escape (*2*). A second is the activation of mechanisms to prevent future harm by learning to recognize signals of impending pain (*3*), allowing future painful events to be expected and thus avoided.

Functional neuroimaging has previously been used to identify cerebral activation patterns associated with the experience of pain (*4*, *5*). Brain areas activated during peripheral painful stimulation included anterior cingulate, insular, prefrontal and somatosensory cortices, and the thalamus (*6*). Attempts to discriminate between brain responses associated with the expectation of pain and those associated with the direct experience of pain are only now beginning (*7*). This distinction is important because not only do these two processes have the separate adaptive consequences outlined above, but they also have potentially separate, maladaptive consequences. For example, expectation of pain by itself may be an important factor in the development of chronic pain syndromes (*8*). A dissection of the functional neuroanatomies of the expectation and the experience of pain could therefore aid development of therapeutic strategies for the treatment of chronic and acute pain.

Twelve healthy volunteers underwent functional magnetic resonance imaging (fMRI) (*9*) while being presented with a pseudo-random sequence of two intensities of thermal stimulation (painful hot or nonpainful warm). Colored lights signaled in advance the two kinds of thermal stimulation. Subjects learned during the imaging session which color signaled pain and which signaled warmth (*10*). We identified brain regions involved in the experience of pain by comparing brain activation during pain with activation during warm stimulation. This comparison, denoted "pain," controls for somatosensory input unrelated to pain. In addition, we identified brain regions involved in the anticipation of pain by comparing brain activation during the colored light preceding pain to activation during the colored light preceding warm stimulation. This comparison, denoted "anticipation," controls for anticipatory processes unrelated to pain (*11*).

Interviews after the experiment confirmed that all subjects were aware of the relation between the light color and the intensity of the thermal stimulation. Subjects rated painful heat significantly higher than nonpainful warmth on two 11-point visual analog scales measuring intensity [mean ± SD, 7.3 ± 1.3

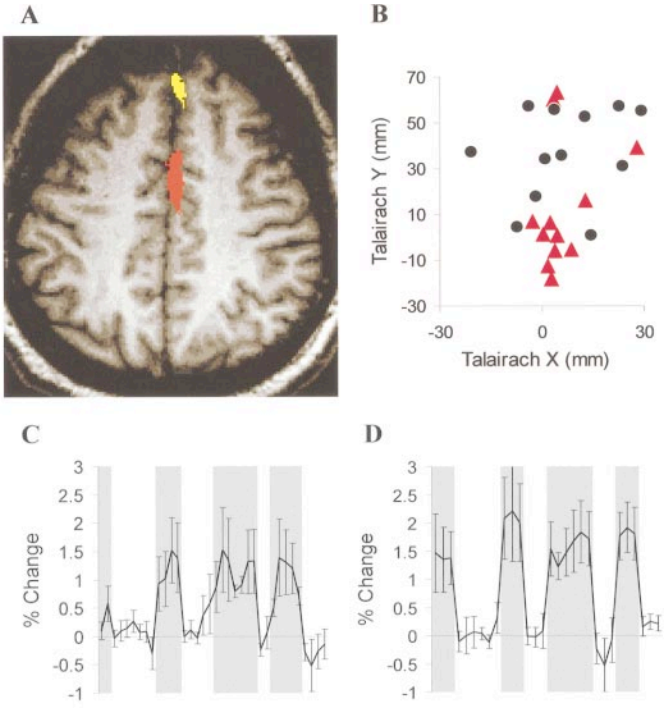

Fig. 1. Medial frontal lobe. (**A**) Group-combined activation map showing volumes selectively activated during pain (red) and anticipation of pain (yellow). (**B**) Individual subject's activation centers during pain (red triangles) and anticipation of pain (black circles). Centers associated with the anticipation of pain (black circles; mean Talairach coordinates $x = 8$ mm, $y = 38$ mm, $z = 27$ mm) were significantly more anterior than those associated with pain [red triangles; mean coordinates $x = 3$ mm, $y = 4$ mm, $z = 33$ mm (*24*)] ($P < 0.05$). (**C**) Time course of fMRI signal intensity change over the period of the scan averaged across subjects. Epochs related to anticipation of pain are shaded in gray (mean ± SEM). (**D**) Time course of fMRI signal intensity change over the period of the scan averaged across subjects (mean ± SEM). Epochs of pain are shaded in gray.

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("moderate-strong pain") versus 2.3 ± 0.9 ("warm, no pain"), $P < 0.01$] and unpleasantness [mean ± SD, 4.9 ± 1.7 ("distressing") versus 1.0 ± 0.2 ("comfortable"), $P < 0.01$] of somatosensory stimulation.

We observed clear activation in brain regions previously reported in neuroimaging studies of pain (*6*, *12*). Crucially, within this network of activation, we identified three brain regions (medial frontal lobe, insular cortex, and cerebellum) where responses to pain could be dissociated from those to the anticipation of pain on the basis of differences in neuroanatomical localization and the time course of the fMRI signal change.

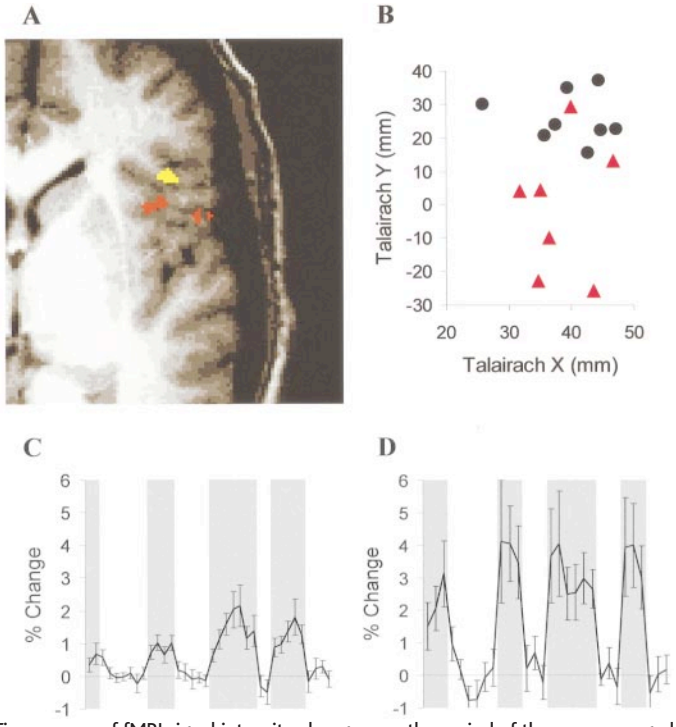

Fig. 2. Insular cortex. (**A**) Group-combined activation map showing volumes selectively activated during pain (red) and anticipation of pain (yellow). (**B**) Individual subject's activation centers during pain (red triangles) and anticipation of pain (black circles). Centers associated with the anticipation of pain (black circles; mean Talairach coordinates x = 40 mm, y = 26 mm, z = 10 mm) were significantly more anterior than those associated with pain (red triangles; mean Talairach coordinates x = 38 mm, y = −1 mm, z = 11 mm) ($P < 0.05$). (**C**) Time course of fMRI signal intensity change over the period of the scan averaged across subjects. Epochs related to anticipation of pain are shaded in gray (mean ± SEM). (**D**) Time course of fMRI signal intensity change over the period of the scan averaged across subjects (mean ± SEM). Epochs of pain are shaded in gray.

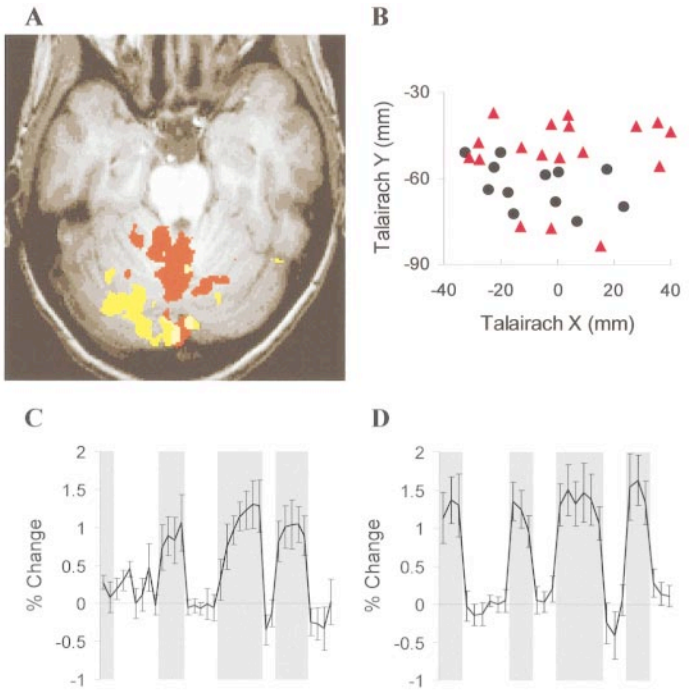

Fig. 3. Cerebellum. (**A**) Group-combined activation map showing volumes selectively activated during pain (red) and anticipation of pain (yellow). (**B**) Individual subject's activation centers during pain (red triangles) and anticipation of pain (black circles). Activation centers appear to form bands, an anterior one associated with pain (red triangles; mean Talairach coordinates x = 3 mm, y = −53 mm, z = −21 mm) and a significantly ($P < 0.05$) more posterior one associated with anticipation of pain (black circles; mean Talairach coordinates x = −7 mm, y = −62 mm, z = −21 mm). An additional small band of pain-related activations is apparent at the cerebellar pole. (**C**) Time course of fMRI signal intensity change over the period of the scan averaged across subjects. Epochs related to anticipation of pain are shaded in gray (mean ± SEM). (**D**) Time course of fMRI signal intensity change over the period of the scan averaged across subjects (mean ± SEM). Epochs of pain are shaded in gray.

The medial frontal lobe was activated in 7 subjects during anticipation of pain and in 10 subjects during pain itself. Both the group analysis (Fig. 1A) and the individual subject analyses (Fig. 1B) showed that pain activated caudal anterior cingulate cortex, whereas the anticipation of pain activated a more anterior region extending from perigenual cingulate to the frontal pole ("anterior medial frontal cortex"). Time courses of the fMRI signal also differed for pain and for its anticipation. The signal associated with the colored light preceding pain (Fig. 1C, shaded area) increased over successive trials (linear trend, $P < 0.05$). In contrast, painful stimuli (Fig. 1D, shaded area) produced a clear fMRI signal on the first trial that remained constant throughout subsequent testing (no significant trends).

Insular cortex was activated in eight subjects during anticipation of pain and in seven subjects during pain itself. Both the group analysis (Fig. 2A) and separate analysis of data from individual subjects (Fig. 2B) showed that activation related to pain was located in the mid-insula, whereas the activation related to the anticipation of pain was found in the anterior insula. The time courses of fMRI signal were again different for pain and for its anticipation. The signal associated with the colored light preceding pain (Fig. 2C, shaded area) increased over trials (linear trend, $P < 0.05$). In contrast, signal amplitude associated with painful stimuli (Fig. 2D, shaded area) remained constant throughout the scanning session (no significant trends).

The cerebellum was activated in 10 subjects during the anticipation of pain and in 9 subjects during the period of the painful stimulation. The group-combined volume of activation (Fig. 3A) associated with pain was localized to the anterior cerebellum and was bilateral; activation associated with the anticipation of pain was localized in posterior cerebellum and was predominantly ipsilateral for data summed across the entire group. The time courses of fMRI signal were again different for pain and its anticipation. The signal associated with the colored light preceding pain (Fig. 3C, shaded area) increased over trials (linear trend, $P < 0.05$), whereas signal amplitude associated with painful stimuli (Fig. 3D, shaded area) was consistent throughout the scanning session (no significant trends).

Dissociations in these brain regions were specific to pain and its anticipation. They were also seen when comparing brain activation during pain to activation during the colored light preceding pain (*13*), but not when contrasting warm stimulation with baseline and anticipation of warm stimulation with baseline (*14*).

Our study demonstrates that the neural substrates of pain and its anticipation can be discriminated both by the involvement of distinct brain regions and the differing response characteristics of these areas (*15*). This conclusion receives substantial support from our finding that anterior medial frontal cortex, anterior insula, and posterior cerebellum did not activate throughout the entire presentation of the colored light associated with pain, but only during the time before onset of the painful stimulus. The experience of pain activated caudal anterior cingulate cortex, mid-insula, and anterior cerebellum ("pain regions"), whereas anticipation of pain activated anterior medial frontal cortex (*16*), anterior insula, and posterior cerebellum ("anticipation regions"). Activation in the pain regions was consistent from trial 1 onward (Figs. 1 to 3D), whereas activation in the anticipation regions increased over trials. Such an increase would be expected as subjects learn that the colored light predicts pain. This indicates that fMRI can monitor processes possibly associated with learning cues to painful events.

Each of the anticipation regions has in close proximity a region mediating pain experience. This arrangement suggests a way in which learning to predict pain may occur by some form of local interaction (*17*, *18*). Perhaps this arrangement allows signals of impending pain to activate different aspects of autonomic [insula (*19*)], affective [medial frontal (*5*, *20*)], and motor [cerebellum (*21*)] function than those that would be activated by pain itself.

Previous functional neuroimaging studies of pain have reported activation of anterior medial frontal cortex and anterior insula during painful stimulation (*6*). Our results show that these activations are not responses to pain itself; rather, they are responses to the anticipation of pain. Previous studies probably contained incidental cues to impending pain and integrated brain activation over longer intervals [using positron emission tomography (PET) rather than fMRI], thereby confounding to varying degrees pain with anticipation (*4*). The present study was designed to separate anticipation and pain temporally by adding explicit cues before thermal stimulation that were designed to overshadow any incidental cues (*22*). This design could be used to evaluate the efficacy of pharmacological or psychological interventions directed specifically at minimizing either responses to pain or to its anticipation. Such an approach might also offer new insights into mechanisms of abnormal sensitivity to pain or of chronic pain syndromes.

References and Notes

1. R. Melzack and K. L. Casey, in *The Skin Senses*, D. R. J. Kenshalo, Ed. (Thomas, Springfield, IL, 1968), pp. 423–443.
2. C. Darwin, *The Expression of the Emotions in Man and Animals* (Murray, London, 1872).
3. I. P. Pavlov, *Conditioned Reflexes* (Oxford Univ. Press, Oxford, 1927); O. H. Mowrer, *Psychol. Rev.* **45**, 62 (1938).
4. A. K. P. Jones, W. D. Brown, K. J. Friston, L. Y. Qi, R. S. Frackowiak, *Proc. R. Soc. London Ser. B.* **244**, 39 (1991); J. D. Talbot *et al.*, *Science* **251**, 1355 (1991); K. L. Casey *et al.*, *J. Neurophysiol.* **71**, 802 (1994).
5. A. D. Craig, E. M. Reiman, A. Evans, M. C. Bushnell, *Nature* **384**, 258 (1996); P. Rainville, G. H. Duncan, D. D. Price, B. Carrier, M. C. Bushnell, *Science* **277**, 968 (1997).
6. J. C. Hsieh *et al.*, *Pain* **64**, 303 (1996); S. W. G. Derbyshire and A. K. P. Jones, *ibid.* **76**, 127 (1998).
7. J.-C. Hsieh, S. Stone-Elander, M. Ingvar, *Neurosci. Lett.* **262**, 61 (1999).
8. J. Lethem, P. D. Slade, J. D. Troup, G. Bentley, *Behav. Res. Ther.* **21**, 401 (1983); N. Birbaumer, H. Flor, W. Lutzenberger, T. Elbert, in *Advances in Pain Research and Therapy*, B. Bromm and J. E. Desmedt, Eds. (Raven, New York, 1995), vol. 22, pp. 331–343; G. Crombez, L. Vervaet, F. Baeyens, R. Lysens, P. Eelen, *Behav. Res. Ther.* **34**, 919 (1996).
9. Twelve right-handed volunteers were studied (seven males, five females; mean ± SD, 26 ± 3 years). All subjects gave informed consent, and the study was approved both by the Oxfordshire Committee for Research Ethics and the University of Western Ontario Ethics Review Board. Data were acquired on a Varian/Siemens 4T whole-body scanner with a birdcage head coil. Head movements were restrained with foam pads. Contiguous slices were prescribed parallel to the AC-PC line and covered the entire brain volume. BOLD contrast multishot echo-planar images were obtained with the following acquisition parameters: TR = 2.5 s, TE = 15 ms, 8-mm slice thickness, field of view = 22 cm by 22 cm, 64 by 64 by 15 matrix. Structural images were obtained with a standard T1-weighted pulse sequence.
10. Noxious and warm thermal stimuli were applied to the dorsum of the left hand with a 3 cm by 3 cm Peltier thermode, designed and built in-house. In the scanner, an adaptive procedure was used to identify two stimuli consistently described by the subject as "painfully hot" and "clearly warm, but not painful." Three color light-emitting diodes (LEDs; red, green, blue) were mounted at the subjects' feet and could be viewed through a mirror in the magnet bore. During the experiment, subjects received five noxious and five innocuous stimulations in pseudo-random (PR) order. Each type of stimulation was consistently signaled by a certain color LED for each subject (randomized across subjects), which preceded the onset of thermal stimulation by a PR interval (mean ± SD, 7.5 ± 5 s) and remained on during the 11 s of thermal stimulation. Between conditioning trials subjects had a rest period of PR duration (mean ± SD, 26.5 ± 9 s) signaled by the third LED. Subjects were instructed to determine the contingency between LED color and thermal stimulation. After the experiment, subjects rated the two thermal stimuli on two 11-point visual analog scales of pain intensity and unpleasantness.
11. Image processing and statistical analysis were carried out with MEDx 3.0 (Sensor Systems). All volumes were realigned, smoothed with a 3.5 mm by 3.5 mm by 5 mm (full width at half maximum) Gaussian kernel, and the average of every volume was normalized to the same mean value. Voxel-by-voxel linear detrending and wavelet temporal filtering were applied. Activation maps were calculated by Student's parametric unpaired t test, spatially normalized to Talairach space, and cluster detection was performed on all voxels above $z = 2$ to determine clusters significantly activated in the experimental task conditions ($P < 0.01$). Group-combined activation maps show cluster volumes common to at least three subjects (Figs. 1 to 3A). In contrast, cluster centers of mass for individual subjects (Figs. 1 to 3B) and group coordinates were derived from data for all individual subjects showing significant activation. Group-averaged time courses (Figs. 1 to 3, C and D) show mean ± SEM of fMRI percent signal change over the period of the scan for the highest z-score voxel within each cluster. Regional shifts in activation were examined for significance by t tests ($P < 0.05$), and time courses were tested for significant linear or quadratic trends by polynomial contrasts analysis of variance ($P < 0.05$).
12. Observed activations included thalamus, basal ganglia, and primary and secondary somatosensory cortices (*23*).
13. Activations for the light preceding pain relative to pain itself [subjects activated (cluster total): medial frontal lobe, 6 (8); insula, 3 (3); cerebellum, 6 (8)] were significantly anterior to activations for pain relative to the light preceding pain [subjects activated (cluster total): medial frontal lobe, 7 (9); insula, 8 (8); cerebellum 6 (11)] in the medial frontal lobe ($P = 0.002$) and the insula ($P = 0.045$), and significantly lateral in the cerebellum ($P = 0.003$). Talairach coordinates for the light preceding pain relative to pain were as follows [x (mm), y (mm), z (mm)]: medial frontal lobe [−7, 47, 16], insula [38, 19, 4], cerebellum [−3, −60, −19]; and for pain relative to the light preceding pain: medial frontal lobe [2, 6, 34], insula [39, 4, 9], cerebellum [−6, −56, −26].
14. No regional shifts in activation between warm stimulation relative to baseline [subjects activated (cluster total): medial frontal lobe, 3 (4); insula, 1 (1); cerebellum 4 (4)] and expectation of warm stimulation relative to baseline [subjects activated (cluster total): medial frontal lobe, 4 (5); insula, 2 (2); cerebellum, 4 (5)] were observed in the medial frontal lobe ($P = 0.51$), the insula ($P = 0.70$), or the cerebellum ($P = 0.83$). Brain activation in these regions during the light preceding warm stimulation relative to baseline appears to be related to an expectation of pain during initial trials of this type, that is, before the contingency between lights and thermal stimuli is learnt. This was confirmed by interview after the experiment and is paralleled by higher fMRI signal intensity on early relative to late presentations of the light preceding warm stimulation (Figs. 1 to 3C).
15. This dissociation contrasts with William James's theory that anticipation depends on activity in the same networks that process the actual experience [W. James, *Text-Book of Psychology* (Macmillan, London, 1892)].
16. This finding is in agreement with an early study of signaled aversive conditioning [A. Elithorn, M. F. Piercy, M. A. Crosskey, *J. Neurol. Neurosurg. Psychiatry* **18**, 34 (1955)]: Patients given prefrontal leucotomy showed reduced psychogalvanic responses related to the anticipation of pain with no change in pain tolerance. Our study suggests a specific neuroanatomical basis for their observations.
17. Coherence of γ-band electroencephalogram activity [W. H. R. Miltner, C. Braun, M. Arnold, H. Witte, E. Taub, *Nature* **397**, 434 (1999)] is an interaction compatible with fMRI signal increase in only one of the participating regions.
18. In the medial frontal cortex the dense opioid receptor population [B. A. Vogt, H. Watanabe, S. Grootoonk, A. K. P. Jones, *Hum. Brain Map.* **3**, 1 (1995)] may support this interaction.
19. J. R. Augustine, *Brain Res. Rev.* **22**, 229 (1996).
20. A. Bechara, H. Damasio, D. Tranel, A. R. Damasio, *Science* **275**, 1293 (1997).
21. J. E. Steinmetz, S. F. Logue, D. P. Miller, *Behav. Neurosci.* **107**, 941 (1993). See G. Allen, R. B. Buxton, E. C. Wong, and E. Courchesne [*Science* **275**, 1940 (1997)] for a nonmotor account.
22. L. J. Kamin, in *Fundamental Issues in Associative Learning*, N. J. Mackintosh and W. K. Honig, Eds. (Dalhousie Univ. Press, Halifax, 1969), pp. 42–64.
23. A. Ploghaus *et al.*, data not shown.
24. The Talairach y coordinate lies anterior to the mean, but within the distribution, of coordinates of anterior cingulate cortex activation reported in PET studies on pain [J.-C. Hsieh, M. Belfrage, S. Stone-Elander, P. Hansson, M. Ingvar, *Pain* **63**, 225 (1995)].

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